



Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.



ELSEVIER

The Science of the Total Environment 154 (1994) 201–115

the Science of the
Total Environment

An International Journal for Scientific Research
into the Environment and its Relationship with Man

Pathology and toxicology of beluga whales from the St. Lawrence Estuary, Quebec, Canada. Past, present and future

D. Martineau^{*a}, S. De Guise^{a,b}, M. Fournier^b, L. Shugart^d, C. Girard^a,
A. Lagacé^a, P. Béland^c

^aDépartement de Microbiologie et Pathologie, Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, J2S 7C6, Canada

^bDépartement des Sciences Biologiques, Université du Québec à Montréal, C.P. 8888, Succ. A, Montréal, Québec, H3C 3P8, Canada

^cInstitut National d'Écotoxicologie du Saint-Laurent, 3872 Parc-Lafontaine, Montréal, Québec, H2L 3M6, Canada

^dEnvironmental Sciences Division, Oak Ridge National Laboratory, PO Box X, Oak Ridge, TN 37831, USA

Abstract

An indigenous population of 450–500 beluga whales (*Delphinapterus leucas*) inhabiting the St. Lawrence Estuary has been exposed chronically for more than 50 years to a complex mixture of industrial pollutants including organochlorinated compounds (OC), polycyclic aromatic hydrocarbons (PAH) and heavy metals. From 1983 to 1990, we have necropsied 45 well preserved carcasses out of a total of 120 beluga whales reported dead over this period. Of these 45 animals, nine were affected by 10 malignant neoplasms. Fifteen animals (33%) were affected by pneumonia. Milk production was compromised in eight of 17 mature females (41%), by inflammatory changes (seven animals) and cancer (one animal) which affected the mammary glands. Opportunistic bacteria were found in pure culture, and/or in significant amounts in at least two organs in 20 belugas (44%). The concentrations of both total PCBs and highly chlorinated PCB congeners were much higher in St. Lawrence animals than in Arctic beluga whales. OC-induced immunosuppression has been repeatedly demonstrated in a wide variety of animal species. Therefore, it is probable that the immune functions of St. Lawrence beluga whales are impaired. Benzo[*a*]pyrene adducts were detected in 10 of the 11 St. Lawrence beluga whales of which tissues (six livers, 10/11 brains) were analyzed by a method based on HPLC. No such adducts were found in four Arctic animals. Since benzo[*a*]pyrene is one of the most potent chemical carcinogens known to man, these compounds might be responsible for some of the cancers observed in that population. Overall, our findings contrast vividly with those of others who found that cancers are exceedingly rare in free-ranging odontocete populations and that the major causes for mortalities in these populations are bacteria, parasites, and trauma.

Keywords: Beluga; Cancer; Cetaceans; Contaminants; DDT; Immunosuppression; Organochlorine; Pathology; PCB; Polycyclic Aromatic; Hydrocarbons; Whales

^{*} Corresponding author.

1. Introduction

The St. Lawrence River Estuary is inhabited by the southernmost population of beluga whales (*Delphinapterus leucas*). This population has dwindled from an estimated 5000 animals to the current estimated 450–500 [1]; it is unique by its small size, geographical isolation, and relative accessibility. The confinement of this population to a basin heavily contaminated by industrial pollutants, the long life span of the species (25–30 years) comparable to that of man, and its life-long exposure to industrial contaminants through diet all contribute to make the St. Lawrence beluga whale population an extraordinary opportunity to demonstrate the impact of chronic exposure to industrial and agricultural contaminants on higher mammals.

The population and the area it inhabits have been the object of extensive scientific scrutiny for a decade; as a result, the toxicological, pathological and social profiles of this population have been characterized and are currently being refined [2–12]. Stranded carcasses are highly contaminated with organohalogens [6] and benzo[a]pyrene DNA adducts have been detected in the brain of some whales [7,13]. Post-mortem examination of these carcasses has revealed a high prevalence of opportunistic infections suggestive of immunosuppression [7]. In addition, several lesions such as squamous metaplasia of mammary glands, and gastric ulcers, consistent with organohalogen toxicity, have been reported [7,14,15]. Squamous metaplasia of mammary glands, without concurrent parasitic infestation, has been observed in another population of cetaceans contaminated with PCBs and an etiological association with PCB toxicity has been also suggested in these animals [16].

The Great Lakes basin is probably one of the best studied ecosystems heavily contaminated by industrial pollutants. The St Lawrence River and Estuary are the end point of that basin and constitute a major link between the North American continent and the North Atlantic waters. The sediments and several aquatic animals of this region have been found to be contaminated by heavy metals such as mercury and lead, by poly-

cyclic aromatic compounds (PAH) and by organohalogen compounds, among which DDT and polychlorinated biphenyls (PCBs) are prominent [6,7,10,11,17–26]. PCB concentrations found in the tissues of some birds and fish are similar to those found in the Great Lakes [27]. Some PCBs are stable and, because of this stability, have persisted in the environment a long time after their production was banned in 1972. Large amounts remain in use in heavy power equipment, at least in the USA. Even larger quantities have been buried in landfills or dumped at sea, e.g. an estimated 240 million kg of PCBs have been dumped into landfills, up to 1972 [28].

2. Material and methods

2.1. Animals

From January 1983 to December 1990, marine mammal mortalities were reported by a network of volunteer and casual observers. Fresh carcasses were transported on ice to the facilities of the Department of Pathology, Faculté de Médecine Vétérinaire de l'Université de Montréal. Age of the whales was determined by counting dentine growth layers on longitudinal section of teeth [29], adopting the standard of two dentine growth layers per year [30].

2.2. Pathology and bacteriology

Lesions and tissues were cultured on blood agar, incubated at 37°C in a 10% CO₂ atmosphere and examined after 48 h. Identification of bacteria was carried out with an Analytical profile Index diagnostic kit (Analytab Products, Ayerst Laboratories, Plainview, NY). For culture of potential *Vibrio* sp., tissue samples were placed on trypticase soy agar (BBL, Cockeysville, MD) with 5% bovine blood and MacConkey agar; the plates were incubated at 37°C for 24 h. Samples of all major organs and lesions were preserved in neutral 10% formalin and fixed tissues were embedded in paraffin, sectioned 5 µm thick and routinely stained with haematoxylin-phloxin-saffron. Special stains were used when necessary.

Control tissue was obtained from beluga whales

killed by native hunters in Nastapooka, Eastern Hudson Bay, and in Eskimo Point, Western Hudson Bay, Pangnirtung, Baffin Island and in Grise Fjord, Ellesmere Island. Tissues were obtained from a captive beluga whale at the Vancouver Aquarium which presumably died of anaphylactic shock.

2.3. Toxicology

Organochlorines (OC)

Analyses were carried out at the Centre d'Océanologie de Rimouski (RKI) and at Freshwater Institute, Fisheries and Oceans Canada, Winnipeg (WPG). Briefly, a wet sample (5–20 g) was homogenized with a Virtis blender (20 000 rev./min) in a glass container. After adding sodium sulphate to completely absorb tissue humidity, the sample was extracted three times in an ultrasonic bath with 100 ml of a 50:50 hexane/dichloromethane mixture. Extracts were combined, reduced to 3–4 cc, and purified on a molecular Bio-beads SX3 (Biorad) sieve as described to eliminate lipids and other large undesired molecules, and finally purified on a silicon column [31]. Quantitation and identification of PCBs were done by gas chromatography on a DB5 capillary column with a flame ionization detector, and confirmed by gas chromatography/mass spectrometry.

Time trends in contaminant levels were discussed by comparing data from this study with samples collected from the same population in 1982–1987 and analyzed at a third laboratory, Laboratoire Capitaine Bernier, Fisheries and Oceans Canada, Longueuil (LGL) [6]. Samples from nine animals were sent to two laboratories, WPG and LGL. Both produced similar results for DDT and metabolites but the LGL laboratory consistently returned higher values (by an average factor of 1.47) for total PCBs as these were assessed on the basis of a mixed Aroclor 1254:1260 standard, as opposed to the summation of individual congener peaks done at WPG. For the purpose of time trend analysis, we adopted a procedure similar to that used by others [32] and we reduced the data for 1983–85, measured at LGL, by a factor of 1.47.

Benzo[a]pyrene DNA adducts

The analysis of benzo[a]pyrene (B[a]P) adducts was carried out at RKI [33]. The methodology was adapted from Shugart et al. 1983 as summarized below [34]. Extraction and purification: 100–200 mg of tissue were homogenized in 3 ml of NH_4OH (1 N) and put into a glass tube containing 10 mg of a pronase solution buffered with 20 mM Tris-HCl (pH 7.8), 20 mM NaCl, 1 mM EDTA and 0.1% laurylsulfate. This solution was agitated and incubated for 4 h at 50°C, transferred to a screw-cap centrifugation tube with 1 ml of Tris-HCl buffer (0.1 M, pH 8), and extracted with 3 ml of a 24:1:25 (v/v) chloroform/isoamyl alcohol/phenol mixture (CIP) in a rotating agitator for 30 min. Phases were separated by centrifugation (Beckman model J21-C) at 4°C for 5 min at 1000 rev./min.

The aqueous phase was re-extracted with 3 ml of CIP and stored in the dark. The two CIP phases were then combined and extracted three times at room temperature, always with 1.5 ml of ethyl ether, and twice with 1.5 ml of ethyl acetate. Phases were separated by centrifugation after each extraction to eliminate the organic phase. For every ml of aqueous solution retrieved (about 14 ml), 177 μl of spermine tetrahydrochloride was added. The solution was vigorously agitated and left aside for 2 h at 4°C. The DNA precipitate was recovered through 30 min at 10 000 rev./min centrifugation at 4°C and resuspended for 30 min at 80°C in 1 ml of 50 mM NaCl buffered with sodium phosphate (5 mM, pH 7.1). The contents were transferred into a polypropylene microtube for a second spermine precipitation.

DNA was recovered after 10 min of 11 000 rev./min centrifugation (Beckman Microfuge B) at room temperature. The precipitate was washed three times with 1 ml sodium acetate (0.3 M) and magnesium acetate (10 mM) solution containing 75% ethanol. The DNA precipitate was resuspended in the phosphate buffer (pH 7.1) with 50 mM of NaCl and stored at 4°C. A 180- μl aliquot of the DNA solution was transferred into a polypropylene microtube; 10 μl of 1.2 N HCl were added and the mixture heated at 80°C for 6 h. The hydrolysed DNA sample was diluted to 3 ml with 20% methanol and passed through a Sep-Pak

C18 microcolumn preactivated with 6 ml of deionized water. The microcolumn was washed twice with 3 ml of 20% methanol, tetrols were recovered with 2 ml of 100% methanol, and sample volume was reduced to 0.5 ml and immediately submitted to high pressure liquid chromatography (HPLC).

DNA quantitation. 100 μ l of the DNA suspension was added to a reaction tube containing 100 μ l of 25 mM NaCl, 5 μ l of SDS (0.2% in 2 mM EDTA), 3 ml of the potassium phosphate (0.2 M, pH 6.9) buffer and 3 μ l of Hoechst 33258 (1 mg/ml) stain. The solution was mixed in a Vortex mixer and kept in the dark for 15 min. Sample fluorescence was measured in a Perkin Elmer MPF-44A spectrofluorometer. The DNA standard solution was prepared from calf thymus DNA.

Adduct quantification. Purified adducts were separated and quantified by HPLC, using a Waters 610 data pump and a Waters 470 fluorescence detector operated with a Waters 600E flow control coupled to a Waters 746 data recorder. A C18 Guard Pak precolumn and a C18 (150 \times 3.9 mm) Nova Pak column were used, with flow set at 1 ml/min and temperature maintained at 50°C by an Eldex column heater. After injection, elution was done in isocratic mode with a 50% methanol mixture in water (v/v). Tetrol (benzo[a]pyrene tetrahydrotetrol) standards were obtained from the Midwest Research Institute, MI as an entirely water soluble white powder. Each standard was prepared so as to obtain a standard reference curve between zero and 100 pg per 15 μ l of injected solution.

2.4. Cancer epidemiology

Only cases that were confirmed histopathologically were included in this study. The crude annual incidence rate for cancer per 100 000 animals (CAR) was roughly estimated by dividing the number of cancer cases by 8 years (1983–1990), by 500 whales (the total population) and by multiplying by 100 000.

$$\frac{\text{Animals with cancer}}{\text{Duration of study}} \times \frac{100\,000 \text{ animals}}{\text{Total population}} = \text{CAR}$$

Accounting for the 3 winter months during which stranded carcasses are not reported (12 months/9 months as correction factor) and for the carcasses found dead but not examined in the post-mortem room, the total number of animals dead with cancer over 8 years (ETC) (1983–1990) would be:

$$\frac{\text{Animals with cancer}}{\text{Examined animals}} \times \text{Total mortality} \\ \times \frac{12 \text{ months}}{9 \text{ months}} = \text{ETC}$$

3. Results and discussion

3.1. Total mortality

From 1983 to 1990, 120 animals were found dead from which 99 were aged by counting dentine layers and from which 45 were examined in the Department of Pathology of the Faculté de Médecine Vétérinaire, Saint-Hyacinthe. The crude annual mortality rate is thus 120/8 = 15 dead animals per year. This number is a strict minimum since no carcasses are recovered in winter (3 months) and most likely some carcasses are not recovered during the rest of the year. The adjusted annual (12-month) mortality rate, accounting for winter months, is 20 animals (15/9 \times 12) (Table 1).

Most likely our sample is representative of the population. This study has been carried out over the last 10 years. Considering the life span of beluga whales (30 years), all whales that were over 20 years old in 1983 have died since. The

Table 1
Total mortality of St. Lawrence beluga whales (1983–1990)

Group	1983	1984	1985	1986	1987	1988	1989	1990	Total
Aged ^a	11	10	13	9	9	16	17	14	99
Not aged ^b	5	2	2	1	2	5	3	1	21
Total	16	12	15	10	11	21	20	15	120

^aAging was carried out by counting dentine layers of sectioned teeth.

^bSome animals were not aged for various reasons: (1) the carcass had no teeth; (2) the head was not with the body or teeth were damaged; (3) only pictures were obtained and/or the carcass was identified by reliable observers.

chances of these carcasses being found and examined are even because thorough surveys from airplanes and boats have shown that these whales live in a restricted range. All carcasses have been found within that range or downstream, as a result of drift [35–37].

In winter, the range of the whales is expanded further downstream into the Gulf. During that period, when climatic conditions are harsher, carcasses are not recovered. However, out of the 120 carcasses, 11 animals were found in early spring (mid-April–mid-May), four of these were necropsied and seven were analyzed for contaminants. These animals showed conditions and contaminant levels not different from those of animals found during the rest of the year (data not shown). In addition, animals weakened by old age and/or chronic diseases such as cancer would be expected to die in higher numbers in winter, and thus the cancer prevalence reported here would be underestimated.

3.2. Pathology

Age structure of stranded animals

The age structure of 99 belugas that could be aged is given in Fig. 1.

Non-neoplastic lesions

The digestive system was the major site of non-neoplastic lesions (14/45 animals with gastric ulceration (31%), three with oral ulcers (7%). In contrast with Arctic animals in which the number of teeth range from 25 to 44, 38 of 73 St. Lawrence animals (52%) in which the teeth were

enumerated had less than 25 teeth (Fig. 2). Fifteen animals (33%) were affected by pneumonia. Milk production was compromised in eight of 17 mature females (41%) by inflammatory changes (seven animals) and cancer (one animal) which affected mammary glands. Opportunistic bacteria were found in pure culture, and/or in significant amounts in at least two organs in 20 belugas (44%).

Cancer

There has been no epidemiological study of cancer in specific populations of marine mammals. To determine disease rate in a population, the population at risk must be determined precisely. Marine mammal populations are notoriously ill-defined and/or widespread. The St. Lawrence beluga whale population stands as an exception in this regard since it is well characterized and has been geographically isolated for 10 000 years.

This whale population has been the object of numerous censuses, many of which have been carried out by different techniques [35–37]. All censuses have provided remarkably similar results, defining precisely the population at risk and thus the denominator used to determine the crude annual rate. Here we compare the cancer rate of St. Lawrence beluga whales to the cancer rate in other animals, including man (Table 3).

Frequency of cancer

Nine animals with 10 malignant tumours (cancers) were recorded over an 8-year period (1983–1990) (Table 2). Out of the 45 whales, 38

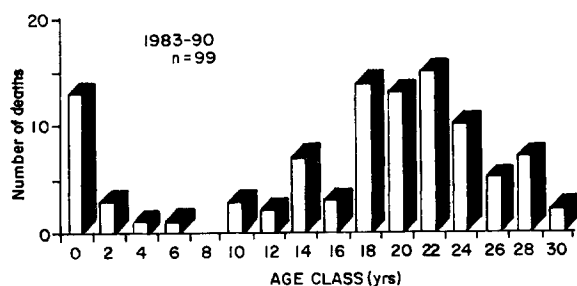


Fig. 1. Age structure of St. Lawrence beluga whales examined from 1983 to 1990.

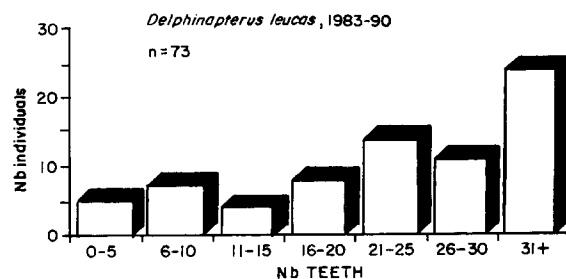


Fig. 2. Number of teeth in St. Lawrence beluga whales examined in 1983–1990 excluding newborn animals (Arctic whales normally have 24–44 teeth).

Table 2
Cancer in stranded beluga whales from the St. Lawrence River 1983–1990

Animal	Sex	Age (years)	Cancer	Reference
DL-18-83	M	16.5	Transitional cell carcinoma	[4]
DL-2-85	F	24.5	Granulosa cell tumor	[7]
DL-6-86	M	24	Salivary gland adenocarcinoma	[95]
DL-4-88	F	21	Gastric adenocarcinoma	[41]
DL-9-88	F	22	Mammary gland adenocarcinoma	[41]
			Hepatocellular carcinoma	[41]
DL-6-89	F	25	Granulosa cell tumor	[41]
DL-7-89	M	29	Intestinal adenocarcinoma ^a	[41]
DL-8-89	M	20	Intestinal adenocarcinoma	[41]
DL-1-90	M	18	Poorly differentiated malignant tumor (liver, mediastinum)	[41]

^aDL-7-89 was the oldest of all animals necropsied.

were mature and thus 24% of necropsied mature animals had malignant neoplasms. The CAR and the ETC were estimated (Table 3):

$$\text{CAR} = \frac{9 \text{ animals with cancer}}{8 \text{ years}} \times \frac{100\,000 \text{ animals}}{500 \text{ animals}}$$

$$= 225 \text{ animals}$$

$$\text{ETC} = \frac{9 \text{ animals}}{45 \text{ animals}} \times 120 \text{ animals} \times 1.33$$

$$= 32 \text{ animals}$$

Thus, 10–32 beluga whales are expected to have died with cancer from 1983 to 1990, an expected CAR of 225–800 animals with cancer per 100 000 animals, respectively. The populations of domestic species used in epidemiologic studies are animals examined at Veterinary Colleges [38]. These animals are probably under better medical

care than the general population and include more sick animals than the general animal population. Advances in veterinary medicine have resulted in more older animals in the population of pet animals [39]. Free-ranging wild animals generally have a shorter life span than captives because of predation, harsh environmental conditions, and malnutrition. Since the risk of developing cancer increases with age, it is reasonable that cancer rates in pet animals would be higher than in free-ranging mammals [40]. Yet, the CAR in our whales is slightly higher than that observed in cattle, and the upper limit of our estimate is similar to the rate observed in dogs. The upper figure for the estimated crude annual incidence of cancer in beluga whales (800/100 000) is higher than that for man.

Table 3
Frequency of cancer in St. Lawrence beluga whales (1983–1990) compared to that of man and domestic animals

Mammal	CAR: Cancer	CAR: Intestinal adenocarcinoma	% of intestinal adenocarcinoma/all cancers	CAR: Ovarian cancer	% of ovarian cancer/all cancers	CAR: liver cancer	% of liver cancer/all cancers
Beluga	225 (800)	50 (175)	20	50 (175)	20	50 (175)	20
Man	363.4	0.69	0.19	10	6	0.8	0.2
Cattle	177.2	1.86	0.26	1.4–110	0.17	5.6	0.5–10
Dog	828.3	6.11	0.28	8.4	0.45	35.7	2
Cat	257.4	13.3	1.8	3.2	0.25	22.8	1.7
Horse	256.3	0	0	16.7	2.9	0.6	0.1
Sheep	0.03	up to 2000	ND	ND	ND	ND	31

CAR: crude annual rate of cancer per 100 000 animals; Parenthesis: adjusted value, accounting for mortality in winter months and for total mortality; References: Man [96,97], Cattle [38,98,99], dog, cat, horse [38,98], sheep [100].

This whale population appears to be affected by a disproportionately high prevalence of cancer. It has to be pointed out that 39% of all tumours reported in cetaceans have been found in St. Lawrence beluga whales, a population of 500 animals [41].

Frequency of specific types of cancer

We found two intestinal adenocarcinomas. This figure corresponds to a crude annual incidence rate of 50 cases/100 000 whales over the whole study (Table 3). Cancers of the small intestine are rare in man and in all domestic animals except in sheep. In the latter, high prevalences (0.2–1.58% of slaughtered sheep) exist in some regions of the world where environmental carcinogens, particularly phenoxy and picolinic acid herbicides, are thought to be a major cause [42].

Two out of the 10 malignant tumours originated from the ovaries. This incidence is higher compared to other animal species except in women where ovarian cancer accounts for 6% of all human cancers if skin cancer is excluded (Table 3).

Table 4

Frequency (%) of cancer by location

Cancer location	Beluga	Man	Cattle	Dog	Cat	Horse
Digestive system	40	22	2.5	0.83–7.7	6.4	2.7
Genital tract	20	30.5	0.17	4.5	0.25	8.9

References: Man [96,97]; cattle, dog, cat, horse [38,98]; sheep [100].

Frequency of cancer by location

The digestive system of beluga whales was more often affected by malignant tumours than that of most domestic animals. Four out of a total of 10 (40%) malignant tumours originated from it. This prevalence resembles that of man where most malignant tumours of the digestive tract occur in the colon and rectum (but rarely in the small intestine) (Table 4).

3.3. Toxicology

Total PCBs

PCB levels in the blubber of St. Lawrence

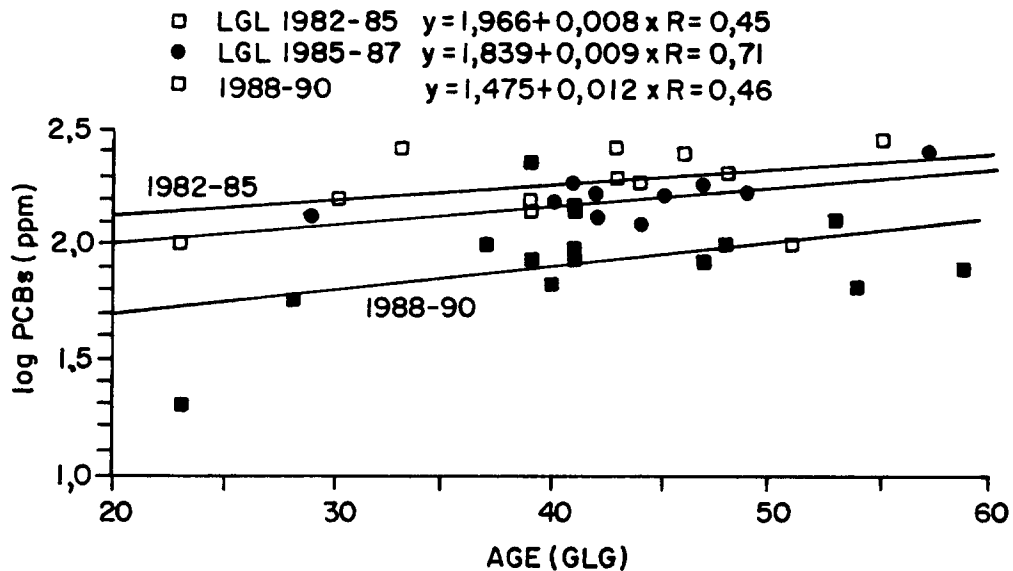


Fig. 3. Trends in blubber PCB levels in adult male St. Lawrence belugas, 1982–1990 (fresh wt.; LGL, WPG and RKI Laboratories). Differences between regressions are not statistically significant, $P > 0.10$.

beluga whales sampled during 1988–90 show the same general characteristics as during the period 1982–1985 (Figs. 3,4) [6]: a logarithmic increase with age, and marked differences between sexes. Data from the later period have higher standard deviations, especially in females, suggesting that this population is more heterogeneous. The data base on PCB levels in St. Lawrence beluga whales, now spanning more than 8 years, suggests a slight progressive decrease in average PCB concentrations in males (Fig. 3). The observed differences between regressions for 1982–1985 and 1985–1987 ($t = 0.167$; $P > 0.10$), and between regressions for 1982–1985 and 1988–1990 ($t = 0.732$; $P > 0.10$), are not statistically significant.

Contamination with benzo[a]pyrene adducts

Benzo[a]pyrene is one of the most powerful chemical carcinogens known by man. This compound has been repeatedly detected in the sediments of the region inhabited by the St. Lawrence beluga whales. The sediments of the Saguenay River contain 500–4500 ppb of PAH (dry wt.) [21]. We have previously demonstrated that there has been a significant interaction between benzo[a]pyrene (B[a]P) and the whale genomic DNA [13,33,43].

It is generally assumed that most carcinogenic

chemicals, or their metabolites, act through covalent binding to DNA. The measure of specific DNA adducts is an accepted means of assessing the degree of interaction between a carcinogen and DNA [44]. DNA adducts resulting from specific exposure to B[a]P were detected in eight of the nine St. Lawrence belugas tested. Adducts were present in six liver tissue samples and in seven of eight brain tissue samples (Table 5). Adduct levels (35–3290 ng/g DNA) in samples where DNA recovery was adequate were higher and more variable than in previous samples (69–206 ng/g DNA) from three animals recovered in 1986 and analyzed at another laboratory [7]. The above data were obtained at two different laboratories, each using one of two methods. A series of analyses at a single laboratory comparing both methods would allow for a more complete interpretation of the observed differences. In comparison, B[a]P DNA adducts were not detected in brain and liver from four Arctic whales (Mackenzie estuary) [13].

Ample evidence that organohalogens have detrimental effects on the immune system of man and animals has been collected over the past two decades. These compounds alter the functions of both arms of the immune system, the cell-mediated immunity and the humoral immunity.

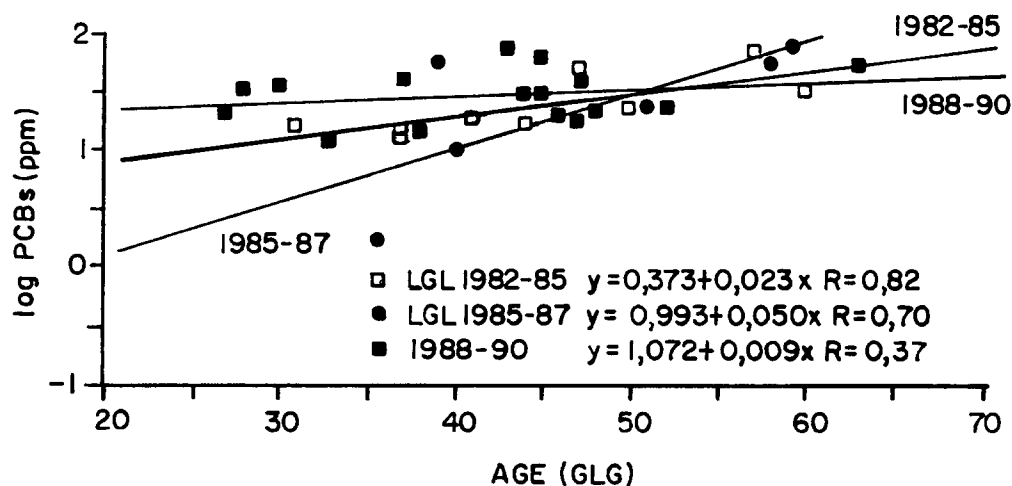


Fig. 4. Trends in blubber PCB levels in adult female St. Lawrence belugas, 1982–1990 (fresh wt.; LGL, WPG and RKI Laboratories).

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD), the most immunotoxic compound, induces thymic atrophy in laboratory animals [45–48]. PCBs, and most notably the coplanar congeners, have similar albeit less severe effects: they cause lymphoid depletion in chicks [47], reduce natural cell cytotoxicity in rat [49,50], decrease the number of T cells and the T helper/T suppressor cells ratio in non-human primates [51] and reduce T cell mediated cytotoxic activity in mice [48].

PCBs decrease antibody production in response to injection of sheep red blood cells (SRBC) in PCBs-treated mice and non-human primates [51–53]. The reduction of serum IgA levels seems to be a consistent component of PCB immunotoxicity [52,54,55]. B cells, and particularly B cell differentiation, are emerging as important targets for halogenated hydrocarbons [45]. In addition, levels of serum corticosteroid, which have a powerful suppressive effect on the immune system and more specifically on T lymphocytes, are altered by PCBs [48,56,57]. Metabolites of PCB are also immunotoxic; chlorinated diphenyl ethers, found in fish from the Great Lakes, decrease significantly the number of circulating lymphocytes in male rats [58].

Not surprisingly, organohalogenes decrease the resistance of laboratory animals to a wide variety of infectious agents: gram-negative bacteria

[53,54], protozoa [54] and viruses [59–61]. Viruses whose virulence is enhanced by organohalogenes include RNA viruses such as duck hepatitis virus (picornavirus) [61], murine leukemia virus (retrovirus) [62], and mouse hepatitis virus (coronavirus) [63] and DNA viruses such as Herpes simplex and ectromelia (Poxvirus) [59]. The complement system, a non-specific defense system against infectious agents, is also altered by PCBs [64].

Immunotoxicity resulting from chronic exposure to organohalogenes has been demonstrated in laboratory and domestic animals but little information exists on free-ranging animals in this regard and data concerning cetaceans are not available. In the past years, reports on stranded cetaceans have appeared in the veterinary literature where organohalogen-induced immunosuppression has been suspected, based on morphological findings and on the observation of opportunistic infections [7,16,65]. However, there have been no systematic efforts to evaluate the impact of pollutants on the immune functions of these animals even though the available data suggest that such an impact might be substantial (tissue concentrations of coplanar PCB congeners, the most toxic PCBs isomers, are higher in cetaceans than in terrestrial animals [66]).

Several reports deal with the histology of lymphoid organs in some species of pinnipeds [67,68]. Antigenic stimulation causes proliferation of lymphocytes and thus hypercellularity of lymph nodes. The absence of antigenic stimulation, like that observed in gnotobiotic animals born by caesarian and kept in germ-free environments, leads to a relative paucity of lymphocytes in the lymph nodes [69–71]. The lymph nodes of pinnipeds from Southern California, an environment highly contaminated with germs, were unexpectedly hypocellular whereas the lymph nodes of Northern fur seals which inhabit the Arctic were more cellular. Since Arctic pinnipeds live in a relatively pristine environment, they are exposed to weaker antigenic stimulations and their lymph nodes were expected to be hypocellular. It has been hypothesized that industrial contaminants caused this hypocellularity [65].

The lack of data regarding PCB-induced immunotoxicity in marine mammals is particularly

Table 5
Benzo[a]pyrene DNA-adducts in brain and liver of St. Lawrence beluga whales

Animal	Sex	Age (years)	Brain adducts (ng/g DNA)	Liver adducts (ng/g DNA)
DL-1-86	F	14	206	ND
DL-2-86	M	22.5	94	ND
DL-4-86	F	17	69	ND
DL-4-88	F	21	8600	710
DL-7-88	M	19	1200	360
DL-8-88	M	20	2000	1320
DL-12-88	F	16	8600	35
DL-3-89	F	19	430	NA
DL-4-89	F	14	NA	780
DL-6-89	F	25	540	NA
DL-7-89	M	29	1290	3290
DL-8-89	M	20	ND	NA

NA, not analyzed; ND, under detection limit.

unfortunate considering (1) that catastrophic viral epidemics have recently affected several populations of marine mammals, all highly contaminated with organohalogenes [72–76]. A possible contribution of organohalogenes to these viral epizootics has been suggested, based on the diminished resistance to viral infections induced by organohalogenes in other animals [77]; (2) that information on the impact of organohalogenes on higher marine life is currently absent from the political and scientific debate on offshore dumping; (3) that considerable efforts and money are invested by individuals and organizations for the rehabilitation of stranded marine mammals whereas the underlying causes for these strandings remain unknown, and finally (4) the endangered status of the St. Lawrence beluga whales [78] and the potential disastrous effects that a viral epidemic would have on this population.

3.4. Future directions

Because we have diagnosed infections consistent with immunosuppression in these animals, we have undertaken a research project to compare the immune functions of St. Lawrence beluga whales to those of less contaminated Arctic populations.

Worldwide, cheetah populations have suffered severe contraction in a short time (population bottleneck). The resulting inbreeding has led to genetically identical individuals. Genetic identity is manifested by the identity of MHC molecules between unrelated cheetahs demonstrated by the lack of rejection of skin transplants [79–81]. Also, probably due to this monoclonality, cheetahs are homogeneously susceptible to some strains of coronaviruses (coronaviruses cause feline infectious peritonitis) that do not seem virulent for other felidae.

Preliminary results obtained by restriction analysis of beluga whale DNA indicate that St. Lawrence beluga whales are genetically more homogeneous than Mackenzie (Canadian Arctic) beluga whales [82]. We hypothesize that the loss of genetic diversity at the MHC locus may be a feature of the St. Lawrence beluga whale popula-

tion since, like cheetahs, this population has suffered a drastic reduction over a short time.

Biomarkers of DNA damage

Detection of oxidative DNA damage. Cancer is one of the ultimate outcomes of genomic DNA damage. It is certainly the most apparent, but unfortunately (for the ecoepidemiologist), it is a rare event and it has a long latency. The development of a solid tumor requires 15–20 years after exposure to a genotoxic compound. Obviously earlier indicators for DNA damage are necessary.

Detection of DNA adducts reflects structural alteration of DNA. However, DNA adducts have a short life and thus their presence reflects only recent exposure. In addition, covalent binding of the chemical or its metabolites to DNA is only one of many ways by which genotoxic contaminants damage DNA. Another mechanism is through microsomal formation of free radicals that can break DNA molecules directly and/or oxidize DNA bases, particularly guanine which is then 8-hydroxylated. Detection of 8-hydroxyguanine in DNA measures oxidative damage in vitro. In vivo, exonucleases release the altered base and the resulting water soluble 8-hydroxydeoxyguanosine (8 OHdG) is excreted (and detected) into urine. The analysis of urine from marine mammals exposed to PAH might demonstrate the presence of this metabolite [83].

Other non-invasive methods of measuring DNA damage have been successfully used in humans. Researchers have looked for increased mutation rates in human blood cells secondary to exposure to carcinogens; the proportion of peripheral blood T lymphocytes that have undergone mutations which render them defective for hypoxanthine-guanine phosphoribosyltransferase (HPRT) reflects the number of mutations presumably induced by carcinogens [84–89]. These methods have an enormous potential in marine mammals since they allow the use of easily accessible biologic material (blood, urine) without sacrificing the animals.

Gross non-specific DNA alterations. If DNA damage caused by genotoxins is not repaired and/or if damage overwhelms repair mechanisms, the

damage may be reflected as variability in the DNA content of affected cells. This variability can be detected by flow cytometry using DNA binding fluorochrome. For instance, hyperdiploid DNA was demonstrated by flow cytometry in hepatocytes from fish from a polluted site while hepatocytes of fish from a reference site had a normal DNA content [90].

Specific DNA damage detected in tumours. Further characterization of DNA damage might be carried out in tumours suspected to have been induced by chemical carcinogens. Spontaneous mutations in genomic DNA are due to the methylation of cytosine in the dinucleotide CpG. The resulting 5-methylcytosine can deaminate spontaneously to thymine causing G:T mismatches which are not always repaired and result in characteristic C to T transitions at CpG sites. This spontaneous mutation leads to 'background mutations' present throughout the genome in all animals.

The p53 tumor suppressor gene is the gene most often altered in human cancer and mutations are distributed over large regions of the gene and include transitions, transversions, and deletions. The variety of site and type of damage found in p53 is explained by the fact that random mutations in tumor suppressor genes are sufficient to inactivate these genes and trigger cancer development. Not surprisingly, DNA from human tumours often show (spontaneous) mutations at a cytosine in dinucleotides CpG randomly distributed in the p53 tumor suppressor gene. This pattern of mutation is found in colon cancer, leukemia, and sarcoma, all tumours that are believed to be truly 'spontaneous'. In contrast, mutations do not follow this pattern of distribution in small cell lung cancers, tumours that are believed to be caused by chemical carcinogens [91]. Similar findings might be obtained from the analysis of tumor DNA in marine mammals. In populations not exposed to chemical carcinogens, most mutations found in spontaneous tumours will most likely affect cytosine in the dinucleotide CpG. In populations exposed to carcinogens, examination of DNA from tumours might reveal that mutations occur at sites that are specific for a given carcinogen.

We have demonstrated the presence of power-

ful carcinogens in St. Lawrence beluga whale tissue along with a high prevalence of tumours. This association suggests a causal relationship between these compounds and cancer. Unfortunately, the number of animals affected with cancer is very small and our findings must be interpreted with caution. The small number of cases is not surprising considering the small size of the population under study and considering that cancer as a result of exposure to carcinogens is a low frequency end point. Convincing statistics would require much larger numbers of animals or the follow-up of this population for many more decades. These are problems which have long been recognized by cancer epidemiologists [92].

The observation of high cancer prevalences in other cetacean populations similarly exposed to carcinogens would considerably strengthen a link with chemical carcinogenesis [93]. Unfortunately, there are only a few concurrent studies of pathologic conditions and tissue contaminant levels in cetacean populations. For these reasons, we stress the importance of undertaking post-mortem inspection of carcasses of marine mammals analyzed for toxic compounds.

In addition, earlier consequences of exposure, more common than cancer, must be used to assess genomic damage by carcinogens. Evidence for genotoxic damage must be gathered: such evidence includes the detection of DNA adducts by the highly sensitive but not very specific ³²P postlabeling technique, the detection of benzo[a]pyrene adducts, the demonstration of high rates of mutations, the presence of unusual mutation patterns, and the demonstration of altered DNA content. Applying these techniques would present relevant information on biodosimetry and estimation of cancer initiation in marine mammal populations exposed to chemical carcinogens [94].

References

- 1 R.R. Reeves and E. Mitchell, Catch history and initial population of white whales (*Delphinapterus leucas*) in the river and Gulf of St. Lawrence, eastern Canada. Nat. Can. (Rev. Ecol. Syst.), 111 (1984) 63–121.
- 2 P. Béland, A. Vezina and D. Martineau, Potential for growth of the St. Lawrence (Quebec, Canada) beluga

- whale (*Delphinapterus leucas*) population based on modelling. J. Cons. Int. Explor. Mer., 45 (1988) 22–32.
- 3 P. Béland, S. De Guise and R. Plante. Toxicology and Pathology of St Lawrence Marine Mammals. Report to the World Wildlife Fund, Inst. Nat. Ecotoxicol. St. Laurent, Montréal, 1991, pp. 1–95.
 - 4 D. Martineau, A. Lagacé, R. Massé, M. Morin and P. Béland, Transitional cell carcinoma of the urinary bladder in a beluga whale (*Delphinapterus leucas*). Can. J. Vet., 26 (1985) 297–302.
 - 5 D. Martineau, A. Lagacé, P. Béland and C. Desjardins, Rupture of a dissecting aneurysm of the pulmonary trunk in a beluga whale (*Delphinapterus leucas*). J. Wildl. Dis., 22 (1986) 289–294.
 - 6 D. Martineau, P. Béland, C. Desjardins and A. Lagacé, Levels of organochlorine chemicals in tissues of beluga whales (*Delphinapterus leucas*) from the St Lawrence Estuary, Quebec, Canada. Arch. Environ. Contam. Toxicol., 16 (1987) 137–147.
 - 7 D. Martineau, A. Lagacé, P. Béland, R. Higgins, D. Armstrong and L.R. Shugart, Pathology of stranded beluga whales (*Delphinapterus leucas*) from the St Lawrence Estuary, Quebec. J. Comp. Pathol., 98 (1988) 287–311.
 - 8 D.C.G. Muir, C.A. Ford, R.E.A. Stewart, T.G. Smith, R. F. Addison, M.E. Zinck and P. Béland, Organochlorine contaminants in belugas, *Delphinapterus leucas*, from Canadian waters. Can. Bull. Fish. Aquat. Sci., 224 (1990) 165–190.
 - 9 S. Ray, B.P. Dunn, J.F. Payne, L. Fancey, R. Helbig and P. Béland, Aromatic DNA-carcinogen adducts in beluga whales (*Delphinapterus leucas*) from the Canadian arctic and the Gulf of St Lawrence. Mar. Pollut. Bull., 22 (1991) 392–396.
 - 10 P. Béland and D. Martineau, in L.D. Shugart (Ed.), Proceedings of the International Conference for Bio-indicators: Exposure and Effects, Oak Ridge National Laboratory, Oak Ridge, Tennessee, 1991.
 - 11 R. Wagemann, R.E.A. Stewart, P. Béland and C. Desjardins, Heavy metals and selenium in tissues of beluga whales, *Delphinapterus leucas*, from the Canadian Arctic and the St Lawrence estuary. Can. Bull. Fish. Aquat. Sci., 224 (1990) 191–206.
 - 12 R. Norstrom, D.C.G. Muir, C.A. Ford, M. Simon, C.R. MacDonald and P. Béland, Classification of P-450 monooxygenase activities in beluga (*Delphinapterus leucas*) from patterns of PCB, PCDD and PCDF accumulation. Mar. Environ. Res., 34 (1992) 267–272.
 - 13 L.R. Shugart, D. Martineau and P. Béland, Detection and quantitation of benzo(a)pyrene adducts in brain and liver tissues of beluga whales (*Delphinapterus leucas*) from the St. Lawrence and Mackenzie estuaries, Presses Université du Québec, Sillery, Québec, 1990, p. 219–223.
 - 14 E. McConnell, J.R. Hass, N. Altman and J. Moore, A spontaneous outbreak of polychlorinated biphenyl (PCB) toxicity in Rhesus monkeys (*Macaca mulatta*): toxicopathology. Lab. Anim. Sci., 29 (1979) 666–673.
 - 15 S. Safe, Polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs): biochemistry, toxicology, and mechanism of action. Crit. Rev. Immunol., 13 (1984) 319–395.
 - 16 D.R. Cowan, W.A. Walker and R.L. Brownell, Jr., in M.M. Bryden and R. Harrison (Eds.), Research on Dolphins, Oxford University Press, London, 1986, Ch. 17, p. 323.
 - 17 Anon., Toxic Chemicals in the Great Lakes and Associated Effects, Environment Canada, Fisheries and Oceans Canada, Health and Welfare Canada, Cat. No. En 37-94/1990-1E, Ottawa, 1991, p. 65.
 - 18 D. Cossa and J.G. Rondeau, Seasonal, geographical and size-induced variability in mercury content of *Mytilus edulis* in an estuarine environment: a reassessment of mercury pollution level in the Estuary and Gulf of St Lawrence. Mar. Biol., 88 (1985) 43–49.
 - 19 M. Castonguay, J.D. Dutil and C. Desjardins, Distinction between American eels (*Anguilla rostrata*) of different geographic origins on the basis of their organochlorine contaminant levels. Can. J. Fish. Aquat. Sci., 46 (1989) 836–843.
 - 20 J.N. Smith and D.H. Loring, Geochronology for mercury pollution in the sediments of the Saguenay fjord, Quebec. Environ. Sci. Technol., 15 (1981) 944–951.
 - 21 L.M. Martel, J. Gagnon, R. Massé, A. Leclerc and L. Tremblay, Polycyclic aromatic hydrocarbons in sediments from the Saguenay fjord, Canada. Bull. Environ. Contam. Toxicol., 37 (1986) 133–140.
 - 22 E. Pelletier, C. Rouleau and G. Canuel, Niveau de contamination par le mercure des sédiments de surface et des crevettes du fjord du Saguenay en 1985–86. Rev. Int. Sci. Eau, 2 (1989) 13–27.
 - 23 N. Goyer. Biphényles polychlorés, Bureau d'étude sur les Substances Toxiques. Ministère de l'Environnement, Gouvernement du Québec, Québec, 1980, p. 18.
 - 24 M. Goulet and D. Laliberté, BPC: Contamination du milieu aquatique au Québec méridional, Direction générale des inventaires et de la recherche. Ministère de l'Environnement, Gouvernement du Québec, Québec, 1982, p. 14.
 - 25 A. Lamarche. Quality of Water for Direct Human Consumption — St. Lawrence Update, Environment Canada, Montreal, 1992, pp. 1–100.
 - 26 M. Paul and D. Laliberté. Teneurs en mercure, plomb, cadmium, BPC et pesticides organochlorés des sédiments et de la chair des poissons du fleuve Saint-Laurent et de la rivière des Outaouais en 1985, Ministère de l'Environnement, Gouvernement du Québec, Québec, 1988, pp. 1–80.
 - 27 J.R. Roberts, D.W. Rodgers, J.R. Bailey and M.A. Rorke, Polychlorobiphenyls: critères biologiques pour évaluer leurs effets sur l'état de l'environnement, Service de publications, CNRC/NRCC, Ottawa, 1979, pp. 148–179.
 - 28 Anon., Panel on hazardous trace substances. Polychlorinated biphenyls — environmental impact. Environ. Res., 5 (1972) 249–362.

- 29 D.E. Sergeant, Biology of white whales (*Delphinapterus leucas*) in western Hudson Bay. J. Fish. Res. Board Can., 30 (1973) 1065–1090.
- 30 P.F. Brodie, The beluga (*Delphinapterus leucas*); growth at age based on a captive specimen and a discussion of factors affecting natural mortality estimates. Rep. Int. Whaling Commn., 32 (1982) 445–447.
- 31 R.J. Norstrom, M. Simon and M.J. Mulvihill, A gel-permeation/column chromatography clean up method for the determination of TCDDs in animal tissue. Int. J. Environ. Anal. Chem., 23 (1986) 267–287.
- 32 R. Turle, R.J. Norstrom and B. Collins, Comparison of PCB quantitation methods: re-analysis of archived specimens of herring gull eggs from the Great Lakes. Chemosphere, 22 (1991) 201–213.
- 33 E. Pelletier, P. Béland and N. Blouin. Développement d'une méthode d'évaluation de l'impact des HAP sur les organismes aquatiques, Inst. Nat. Ecotoxicol. St-Laurent, Contr.: KA 313-9-3949, Montréal, 1990, pp. 1–22.
- 34 L.R. Shugart, J.M. Holland and R.O. Rahn, Dosimetry of PAH skin carcinogenesis: covalent binding of benzo(a)pyrene to mouse epidermal DNA. Carcinogenesis, 4 (1983) 195–198.
- 35 R. Michaud, Distribution estivale du béluga du Saint-Laurent; synthèse 1986 à 1992. Rapp. tech. can. sci. halieut. aquat. 1906, Pêches et Océans Canada, Ottawa, 1993, p. 15.
- 36 M.C.S. Kingsley and M.O. Hammill, Photographic Census Surveys of the St. Lawrence Population, 1988 and 1990. Rapp. tech. can. sci. halieut. aquat. 1776, Pêches et Océans Canada, Ottawa, 1991, pp. 1–19.
- 37 R. Michaud, A. Vézina, N. Rondeau and Y. Vigneault, Distribution annuelle et caractérisation préliminaire des habitats du béluga, *Delphinapterus leucas*, du Saint-Laurent. Rapp. tech. can. sci. halieut. aquat. 1757, Pêches et Océans Canada, Ottawa, 1990, pp. 1–31.
- 38 W.A. Priester and F.W. McKay. The Occurrence of Tumors in Domestic Animals. National Cancer Institute. Monograph 54, U.S. Department of Health and Human Services. Public Health Service. National Institutes of Health, Washington, DC, 1980, pp. 1–210.
- 39 C.R. Dorn and W.A. Priester, in G.H. Theilen and B.R. Madewell (Eds.), Veterinary Cancer Medicine, Lea & Febiger, Philadelphia, PA, 1987, Ch. 3, p. 27.
- 40 M.E. Fowler, in G.H. Theilen and B.R. Madewell (Eds.), Veterinary Cancer Medicine, Lea and Febiger, Philadelphia, PA, 1987, Ch. 24, p. 649.
- 41 S. De Guise, A. Lagacé and P. Béland, Tumors in twenty-four St. Lawrence beluga whales (*Delphinapterus leucas*). Vet. Pathol., 31 (1994) 444–449.
- 42 K.W. Newell, A.D. Ross and R.M. Renner, Phenoxy and picolinic acid herbicides and small intestinal adenocarcinoma in sheep. Lancet (1984) 1301–1305.
- 43 P. Béland, S. De Guise, C. Girard, A. Lagacé, D. Martineau, R. Michaud, D.C.G. Muir, R.J. Norstrom, E. Pelletier, S. Ray and L.R. Shugart, Toxic compounds and health and reproductive effects in St. Lawrence beluga whales. J. Great Lakes Res., 19 (1993) 766–775.
- 44 W.K. Lutz, In vivo covalent binding of organic chemicals to DNA as a quantitative indicator of chemical carcinogenesis. Mutat. Res., 65 (1979) 289–356.
- 45 D. Davis and S. Safe, Immunosuppressive activities of polychlorinated dibenzofuran congeners: quantitative structure-activity relationship and interactive effects. Toxicol. Appl. Pharmacol., 94 (1988) 141–149.
- 46 A. Poland and J.C. Knutson, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and related halogenated hydrocarbons: examination of the mechanism of toxicity. Annu. Rev. Pharmacol. Toxicol., 22 (1982) 517–554.
- 47 L. Andersson, E. Nikolaidis, B. Brunstrom, A. Bergman and L. Dencker, Effects of polychlorinated biphenyls with Ah receptor affinity on lymphoid development in the thymus and the bursa of Fabricius of chick embryos in ovo. Toxicol. Appl. Pharmacol., 107 (1991) 183–188.
- 48 N.I. Kerkvliet, L. Baecher-Steppan, B.B. Smith, J.A. Youngberg, M.C. Henderson and D.R. Buhler, Role of the Ah locus in suppression of cytotoxic T lymphocyte activity by halogenated aromatic hydrocarbons (PCBs and TCDD): structure-activity relationships and effects in C57Bl/6 mice congenic at the Ah locus. Fundam. Appl. Toxicol., 14 (1990) 532–541.
- 49 R.J. Smialowicz, J.E. Andrews, M.M. Riddle, R.R. Rogers, R.W. Luebke and C.B. Copeland, Evaluation of the immunotoxicity of low level PCB exposure in the rat. Toxicology, 56 (1989) 197–211.
- 50 J.H. Exon, P.A. Talcott and L.D. Koller, Effect of lead, polychlorinated biphenyls and cyclophosphamide in rat natural killer cells, interleukin 2, and antibody synthesis. Fundam. Appl. Toxicol., 5 (1985) 158–164.
- 51 H. Tryphonas, S. Hayward, L. O'Grady, J.C.K. Loo, D.L. Arnold, F. Bryce and Z.Z. Zawidzka, Immunotoxicity studies of PCB (Aroclor 1254) in the adult Rhesus (*Macaca mulatta*) monkey — preliminary report. Int. J. Immunopharmacol., 11 (1989) 199–206.
- 52 L.D. Loose, K.A. Pittman, K.F. Benitz and J.B. Silkworth, Polychlorinated biphenyl and hexachlorobenzene induced humoral immunosuppression. J. Reticuloendoth. Soc., 22 (1977) 253–271.
- 53 P.T. Thomas and R.D. Hinsdill, Effect of polychlorinated biphenyls on the immune response of rhesus monkeys and mice. Toxicol. Appl. Pharmacol., 44 (1978) 41–51.
- 54 L.D. Loose, K.A. Pittman, K.-F. Benitz, J.B. Silkworth, W. Mueller and F. Coulston, Environmental chemical-induced immune dysfunction. Ecotoxicol. Environ. Saf., 2 (1978) 173–198.
- 55 N. Shigematsu, Y. Norimatsu, T. Ishibashi, M. Yoshida, S. Suetsugu, T. Kawatsu, T. Ikeda, R. Saito, S. Ishimaru, T. Shirakusa, M. Kido, K. Emori and H. Toshimitsu, Clinical and experimental studies on impairment of respiratory organs in chlorobiphenyl poisoning. Fukuoka Acta Med., 62 (1971) 150.
- 56 D. Wasserman, M. Wasserman, S. Cucos and M. Djavan-

- herian, Function of adrenal gland zona fasciculata in rats receiving polychlorinated biphenyls. *Environ. Res.*, 6 (1973) 334–338.
- 57 S.K. Durham and A. Brouwer, 3,4,3',4'-tetrachlorobiphenyl distribution and induced effects in the rat adrenal gland. Localization in the zona fasciculata. *Lab. Invest.*, 62 (1990) 232–239.
 - 58 I. Chu, D.C. Villeneuve, V. Secours and V.E. Valli, Toxicological assessment of chlorinated diphenyl ethers in the rat. *J. Environ. Sci. Health*, 25 (1990) 225–241.
 - 59 J. Imanishi, H. Nomura, M. Matsubara, M. Kita, S.-J. Won, T. Mizutani and T. Kishida, Effect of polychlorinated biphenyl on viral infections in mice. *Infect. Immunol.*, 29 (1980) 275–277.
 - 60 L.D. Koller and J.E. Thigpen, Biphenyl-exposed rabbits. *Am. J. Vet. Res.*, 34 (1973) 1605–1606.
 - 61 M. Friend and D.O. Trainer, Polychlorinated biphenyl: interaction with duck hepatitis virus. *Science*, 170 (1970) 1314–1316.
 - 62 L.D. Koller, Enhanced polychlorinated biphenyl lesions in Moloney leukemia virus-infected mice. *Clin. Toxicol.*, 11 (1977) 107–116.
 - 63 K. Krzystyniak, J. Bernier, P. Hugo and M. Fournier, Suppression of MHV-3-activated macrophages by dieldrin. *Biochem. Pharmacol.*, 35 (1986) 2577–2586.
 - 64 K.L. White, H. Lysy, J.A. McCoy and A.C. Anderson, Modulation of serum complement levels following exposure to polychlorinated dibenzo-*p*-dioxins. *Toxicol. Appl. Pharmacol.*, 84 (1986) 209–219.
 - 65 J.O. Britt and E.B. Howard, in E.B. Howard (Ed.), *Pathobiology of Marine Mammal Diseases*, CRC Press, Boca Raton, FL, 1983, Ch. 4, p. 80.
 - 66 N. Kannan, S. Tanabe, M. Ono and R. Tatsukawa, Critical evaluation of polychlorinated biphenyl toxicity in terrestrial and marine mammals: increasing impact of non-*ortho* and mono-*ortho* coplanar polychlorinated biphenyls from land to ocean. *Arch. Environ. Contam. Toxicol.*, 18 (1989) 850–857.
 - 67 R.Z. Cavagnolo, The immunology of marine mammals. *Dev. Comp. Immunol.*, 3 (1979) 245–257.
 - 68 J.G. Simpson and M.B. Gardner, in S.H. Ridgway (Ed.), *Mammals of the Sea: Biology and Medicine*, Thomas, Springfield, IL, 1972, Ch. 5, p. 298.
 - 69 G.L. Waxler and D.T. Drees, Comparison of body weights, organ weights and histological features of selected organs of gnotobiotic, conventional and isolator-reared contaminated pigs. *Can. J. Comp. Med.*, 36 (1972) 265–274.
 - 70 T.D. Luckey, *Germfree Life and Gnotobiology*, Academic Press, New York, 1963, p. 360.
 - 71 G.J. Thorbecke, Some histological and functional aspects of lymphoid tissue in germfree animals. *Ann. N.Y. Acad. Sci.*, 78 (1959) 237–246.
 - 72 J.R. Geraci, D.J. St. Aubin, I.K. Barker, R.G. Webster, V.S. Hinshaw, W.J. Bean, H.L. Ruhnke, J.H. Prescott, G. Early, A.S. Baker, S. Madoff and R.T. Schooley, Mass mortality of harbor seals: pneumonia associated with influenza A virus. *Science*, 215 (1982) 1129–1131.
 - 73 S. Kennedy, J.A. Smyth, P.F. Cush, M. McAliskey, S.J. McCullough and B.K. Rima, Histopathologic and immunocytochemical studies of distemper in harbor porpoises. *Vet. Pathol.*, 28 (1991) 1–7.
 - 74 M. Domingo, J. Visa, M. Pumarola, A.J. Marco, L. Ferrer, R. Rabanal and S. Kennedy, Pathologic and immunocytochemical studies of morbillivirus infection in striped dolphins (*Stenella coeruleoalba*). *Vet. Pathol.*, 29 (1992) 1–10.
 - 75 M. Domingo, L. Ferrer, M. Pumarola, A. Marco, J. Plana, S. Kennedy, M. McAliskey and B. Rima, Morbillivirus in dolphins. *Nature*, 348 (1990) 21.
 - 76 P.J. Duignan and J.R. Geraci, Epizootiology of marine mammal morbilliviruses in the western north Atlantic. Tenth Biennial Conference on the Biology of Marine Mammals, Galveston. 11–15 November, 1993.
 - 77 D. Eis, Simplification in the etiology of recent seal deaths. *Ambio*, 18 (1989) 144.
 - 78 F.R. Cook and D. Muir, The committee on the status of endangered wildlife in Canada (COSEWIC): history and progress. *Nat. Can. (Rev. Ecol. Syst.)*, 98 (1984) 63–70.
 - 79 S.J. O'Brien, D. Goldman, C.R. Merrill and M. Bush, The cheetah is depauperate in genetic variation. *Science*, 221 (1983) 459–461.
 - 80 S.J. O'Brien, M.E. Roelke, L. Marker, A. Newman, C.A. Winkler, D. Meltzer, L. Colly, J.F. Evermann, M. Bush and D.E. Wildt, Genetic basis for species vulnerability in the cheetah. *Science*, 227 (1985) 1428–1434.
 - 81 N. Yuhki and S.J. O'Brien, DNA variation of the mammalian major histocompatibility complex reflects genomic diversity and population history. *Proc. Natl. Acad. Sci.*, 87 (1990) 836–840.
 - 82 N. Patenaude and B. White, Reduced genetic variability of the Saint Lawrence beluga whale population as assessed by DNA fingerprinting. *Mol. Ecol.* 3 (1994) in press.
 - 83 S.K. Loft, M. Vistisen, A. Wertz, K. Tjonneland, K. Overvad and H.E. Poulsen, Oxidative DNA damage estimated by 8-hydroxydeoxyguanosine excretion in humans: influence of smoking, gender and body mass index. *Carcinogenesis*, 13 (1992) 2241–2247.
 - 84 D.M. Zimmer, C.S. Aaron, J.P. O'Neill and R.J. Albertini, Enumeration of 6-thioguanine-resistant T-lymphocytes in the peripheral blood of nonhuman primates (*Cynomolgus* monkeys). *Environ. Mol. Mutagenesis*, 18 (1991) 161–167.
 - 85 J.A. Nicklas, T.C. Hunter, J.P. O'Neil and R.J. Albertini, Molecular analyses of in vivo hprt mutations in human T-lymphocytes. III. Longitudinal study of hprt gene structural alterations and T-cell clonal origins. *Mutat. Res.*, 215 (1989) 147–160.
 - 86 R.J. Albertini, J.A. Nicklas and J.P. O'Neil, Studies of DNA alterations in in vivo somatic cell mutations in humans. *Basic Life Sci.*, 53 (1990) 315–328.
 - 87 R.J. Albertini, J.P. O'Neill, J.A. Nicklas, L. Recio and T.R. Skopek, hprt mutations in vivo in human T-lymphocytes: frequencies, spectra and clonality. *Prog. Clin. Biol. Res.*, 340C (1990) 15–24.

- 88 P. Ostrosky-Wegman, R. Montero, A. Palao, C. Cortinas de Nava, F. Hurtado and R.J. Albertini, 6-Thioguanine-resistant T-lymphocyte autoradiographic assay. Determination of variation frequencies in individuals suspected of radiation exposure. *Mutat. Res.*, 232 (1990) 49–56.
- 89 J.P. O'Neil, L.M. Sullivan and R.J. Albertini, In vitro induction, expression and selection of thioguanine-resistant mutants with human T-lymphocytes. *Mutat. Res.*, 240 (1990) 135–142.
- 90 N.K. Jenner, G.K. Ostrander, T.J. Kavanagh, J.C. Livesey, M.W. Shen, S.C. Kim and E.H. Holmes, A flow cytometric comparison of DNA content and glutathione levels in hepatocytes of English sole (*Parophrys vetulus*) from areas of differing water quality. *Arch. Environ. Contam. Toxicol.*, 19 (1990) 807–815.
- 91 P.A. Jones, J.D. Buckley, B.E. Henderson, R.K. Ross and M.C. Pike, From gene to carcinogen: a rapidly evolving field in molecular epidemiology. *Cancer Res.*, 51 (1991) 3617–3620.
- 92 D. Ozonoff and M.P. Longnecker, Epidemiologic approaches to assessing human cancer risk from consuming aquatic food resources from chemically contaminated water. *Environ. Health Perspect.*, 90 (1991) 141–146.
- 93 G.A. Fox, Practical causal inference for ecoepidemiologists. *J. Toxicol. Environ. Health*, 33 (1991) 359–373.
- 94 R.H. Jensen, W.L. Bigbee and R.G. Langlois, Multiple endpoints for somatic mutations in humans provide complementary views for biodosimetry, genotoxicity and health risks. *Prog. Clin. Biol. Res.*, 340C (1990) 81–92.
- 95 C. Girard, A. Lagacé, R. Higgins and P. Béland, Adenocarcinoma of the salivary gland in a beluga whale (*Delphinapterus leucas*). *J. Vet. Diagn. Invest.*, 3 (1991) 264–265.
- 96 Anonymous, Health Reports. Suppl. No.8. Vol. 3. No. 2., Cancer in Canada. 1985, 1986, Statistics Canada, Canadian Centre for Health Information, Ottawa, 1991.
- 97 Anonymous, Statistiques canadiennes sur le cancer, 1992, Institut national du cancer du Canada, Toronto, Canada, 1992, pp. 1–65.
- 98 W.A. Priester and N. Mantel, Occurrence of tumors in domestic animals. Data from 12 United States and Canadian colleges of veterinary medicine. *J. Natl. Cancer Inst.*, 47 (1971) 1333–1344.
- 99 D. Bristol, K.H. Baum and L.E. Mezza, Adenocarcinoma of the jejunum in two cows. *J. Am. Vet. Med. Assoc.*, 185 (1984) 551–553.
- 100 G. Georgsson and H. Vigfusson, Carcinoma of the small intestine of sheep in Iceland. A pathological and epizootiological study. *Acta Vet. Scand.*, 14 (1973) 392–409.